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Mutations as Trapdoors: The Rop-Dimer with Two Competing Native Conformations

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Conformational transitions are the molecular mechanism for regulating protein function. Structure-based models are a computational tractable way to simulate these transitions. A model able to accommodate multiple folding basins is proposed to explore the mutational effects in the folding of the Rop-dimer (ROP). In experiments, ROP mutants show unusually strong increases in folding rates with marginal effects on stability. We investigate the possibility of two competing conformations representing a parallel (P) and the wild-type (WT) anti-parallel (AP) arrangement of the monomers as possible native conformations. We observe occupation of both distinct states and characterize the transition pathways. An interesting observation from the simulations is that, for equivalent energetic bias, the transition to the P basin (non WT basin) shows a lower free-energy barrier. Thus the rapid kinetics observed in experiments appears to be the result of two competing states with different kinetic behavior, triggered upon mutation by the opening of a trapdoor arising from the Rop-dimers symmetric structure. The general concept of having competing conformations for the native state goes beyond explaining ROP's mutational behaviors and can be applied to other systems. A switch between competing native structures might be triggered by external factors to allow, for example, allosteric control or signaling.

1 Introduction

The funneled energy-landscape and the principle of minimal frustration explain protein folding as a diffusive process. Multiple routes lead from the unfolded to the folded state.[1,2] Evolutionary pressure smoothened the underlying energy-landscape sufficiently that local minima or roughness do not interfere with folding. The resulting bias towards the native state is robust, so that changes in environmental conditions or limited mutations change neither the structure of a protein nor its folding behavior.

Structure-based simulations, based on the work of Go[3], use these ideas and stipulate that folding can be simulated *in-silico* by only taking native interactions into account[2,4]. Commonly, one coarse-grained the description of a protein in such simulations. Each amino-acid is described as a single bead centered on the position of the C_α-atoms.

2 Simulations on the Rop-dimer

ROP (repressor of primer) [a] is a homodimer of 2x63 amino acids(AA). It is part of a genetic control mechanism in the ColE1 plasmid system and binds to RNA. Its wild-type (WT) structure is that of a coiled-coil helix bundle with each monomer consisting of two helices (see Fig.). The two monomers are arranged anti-parallelly (AP) for the WT. In a series of experiments, the hydrophobic interface between the two monomers has been

[a] ROP is sometimes also called ROM (Regulator of RNA I).

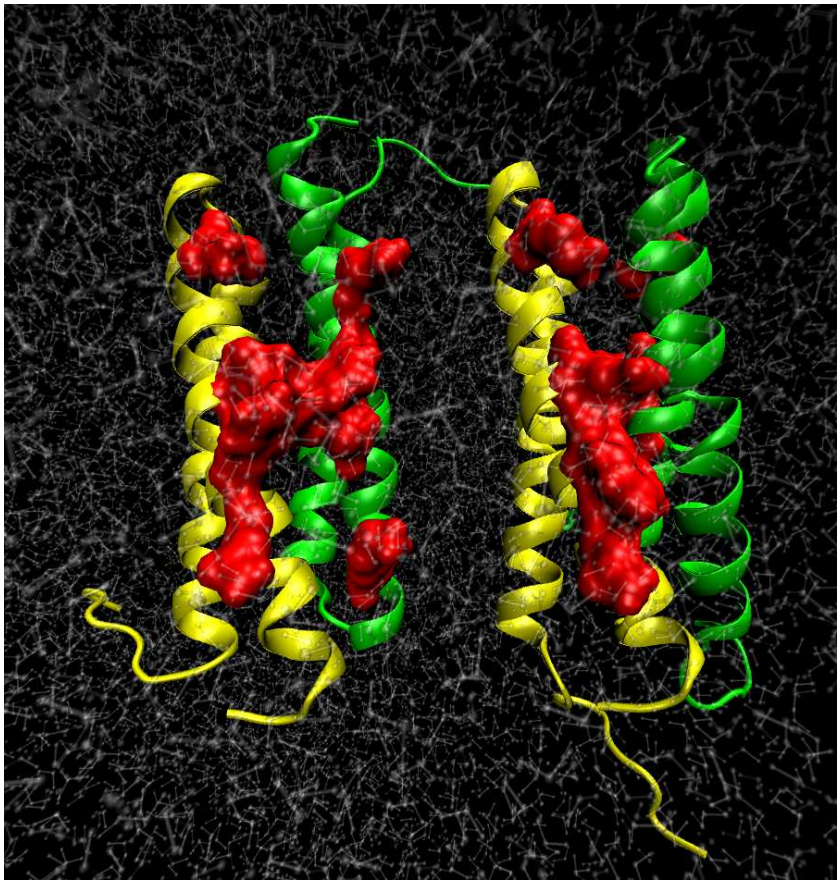

Figure 1. **Structures and symmetry of the Rop-dimer**
Hydrophobic core mutations have a strong effect on the folding/unfolding kinetics and RNA-binding ability of the Rop-homodimer. They can change the WT anti parallel arrangement (left) of monomers (in green and yellow) to a parallel one (right). This conformational transition disrupts the charged RNA-binding interface (highlighted red sidechains) and makes the parallel mutant dysfunctional. A dual-funneled energy landscape with two competing native structures explains Rop's mutational behavior.

mutated.[5,6] These mutations resulted in strong changes of up to four order of magnitude in the folding/unfolding rates. Specific mutants lost their RNA-binding ability, with partly discrepant behavior in vivo and in vitro.[7] One specific mutant has parallelly (P) arranged monomers and lost its RNA-binding ability. This is not surprising, as the ability to bind RNA is linked to an interface on the surface of the WT, which is disrupted in the P structure.

To better understand these experimental results, we assume that the mutations trigger the competition of P and AP by symmetrizing the interface[8,9]. We set up structure-based simulations with two competing native states[9] and observe transition between the unfolded (U), folded P and and folded AP states. It is important to note that we gave both P and AP an exactly equal energetic bias.

In the simulations we observe transitions from U to P and AP. These simulations allow

to derive a free-energy landscape. The free-energy barrier for the transition from U to P is roughly 20% smaller than the one from U to AP. Therefore the kinetics U-P are faster than for U-AP. This is a purely entropic effect resulting from the geometrically more accessible P conformation. In P the less mobile turns of the two monomers can face each other in the transition state ensemble, while the floppy tails of the monomers can still move freely. However in AP the turn face the floppy tail of the other monomer and are therefore more difficult to localize.[9]

ROP's accelerated kinetics for the mutants can therefore be understood as a result from the competition of P and AP. While in the WT the slow kinetics of AP dominate, the mutations in the hydrophobic core open the trapdoor to P and enable P as an off-pathway kinetic trap. One measures an increase in kinetics. [b]

It seems possible, that some mutants possess a degenerated native state, in which both P and AP are present. Especially the mutant $Ala_2Leu_2 - 6$ might express this behavior, as it both binds RNA but is also highly similar to the mutant $Ala_2Ile_2 - 6$, which only differs by possessing Ile instead of Leu for some AA in the hydrophobic core. Current experiments verify this prediction.

Mutant	Binds RNA *in*		Relative		Structure
	vitro	*vivo*	k_F	k_U	
WT	Y	Y	1	1	AP (X-Ray, NMR)
$Ala_2Leu_2 - 2$	Y	Y	3.2	18	AP (*in vitro* activity)
$Ala_2Leu_2 - 4$	Y	P	1.5	28	AP (*in vitro* activity)
$Ala_2Leu_2 - 6$	Y	N	310	31000	AP (*in vitro* activity)
$Ala_2Leu_2 - 8$	Y	N	610	50000	AP (*in vitro* activity)
$Ala_2Leu_2 - 6$-rev	Y	-	85	670	AP (*in vitro* activity)
$Ala_2Leu_2 - 8$-rev	Y	N	92	2700	AP (*in vitro* activity)
$Leu_2Ala_2 - (2+7)$	Y	-	10	18	AP (*in vitro* activity)
$Ala_2Ile_2 - 6$	N	N	-	-	P

Table 1. *Experimental data of the Rop-dimer and some mutants.*
The RNA-binding ability is present (Y), non-present (N) or partially present (P). The folding and unfolding rates k_F and k_U are given relative to the WT. The mutants are named according to the number and location of mutated hydrophobic core amino acid pairs.[5,6] The structures haven been determined only for the WT and the last mutant, all other structures have been assumed to be AP because of their RNA binding behavior.

Acknowledgments

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[b]The kinetics are determined experimentally by Circular Dichroism measurements which cannot distinguish between transitions from U to P or AP, as both states have equivalent helical content.

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